CLAIMS

1. A cell line comprising a stably integrated recombinant nucleic acid construct comprising:

a reporter gene operably linked to a recognition sequence for a sequence-specific DNA-binding protein; and

a stably integrated recombinant nucleic acid construct comprising a sequence encoding a fusion protein, the fusion protein comprising a sequence-specific DNA binding domain, wherein the DNA binding domain specifically binds said recognition sequence, and a conditionally active transactivation domain, wherein activation of said conditionally active transactivation domain is dependent on protein phosphorylation and/or protein:protein interaction, and wherein binding of said fusion protein to said recognition sequence results in transactivation of said reporter gene when said transactivation domain fused to said DNA binding domain is activated.

2. The cell line of claim 1, wherein said reporter gene is selected from the group consisting of luciferase, β-galactosidase, chloramphenicol acetyltransferase, secreted alkaline phosphatase and green fluorescent protein.

3. The cell line of claim 2, wherein said reporter gene is luciferase.

4. The cell line of claim 1, wherein said recognition sequence for a sequence-specific DNA-binding domain is that sequence recognized by one of the group consisting of GAL4

and LexA.

5. The cell line of claim 1 wherein said fusion protein is constitutively expressed.

6. The cell line of claim 1 wherein said fusion protein is constitutively expressed in a specific cell type.

7. The cell line of claim 1, wherein the cell line is of mammalian origin.

8. The cell line of claim 1, wherein said parent cell line is human.

9. The cell line of claim 1, wherein said parent cell line is HeLa.

10. A method of assaying for the activity of a signal transduction pathway in a mammalian cell, said method comprising the steps of:

detecting in a signal transduction pathway-specific reporter cell line expression of a reporter gene, wherein said reporter cell line comprises:

a reporter gene operably linked to a recognition sequence for a sequence-specific DNA-binding protein; and

a stably integrated recombinant nucleic acid construct comprising a sequence encoding a fusion protein, said fusion protein comprising a sequence-specific DNA binding domain, wherein said DNA binding domain specifically binds said recognition sequence,

and a conditionally active transactivation domain, wherein activation of said conditionally active transactivation domain is dependent on protein phosphorylation and/or protein:protein interaction, wherein binding of said fusion protein to said recognition sequence results in transactivation of said reporter gene when said transactivation domain fused to said DNA binding domain is activated, wherein expression of said reporter gene is indicative of activity of said signal transduction pathway.

11. A method of screening for a modulator of the activation of a signal transduction pathway in a mammalian cell, said method comprising the steps of:

(a) contacting a stable reporter cell line with a candidate modulator under conditions sufficient to permit activation of said signal transduction pathway, the reporter cell line comprising:

a reporter gene operably linked to a recognition sequence for a sequence-specific DNA-binding protein; and

a stably integrated recombinant nucleic acid construct comprising a sequence encoding a fusion protein, said fusion protein comprising a sequence-specific DNA binding domain, wherein said DNA binding domain specifically binds said recognition sequence, and a conditionally active transactivation domain, wherein activation of said conditionally active transactivation domain is dependent on protein phosphorylation and/or protein:protein interaction, wherein binding of said fusion protein to said recognition sequence results in transactivation of said reporter gene when said transactivation domain fused to said DNA binding domain is activated; and

(b) detecting expression of said reporter gene, wherein a difference in expression of said reporter gene in the presence of said candidate modulator and in the absence of said candidate modulator is indicative of modulatory activity of said candidate modulator on said pathway.

12. The method of claim 11, further comprising, during said contacting step, providing an activator signal, said signal activating said conditionally active transactivation domain.

13. The method of claim 12, wherein said providing comprises adding an activator compound to the culture medium of said reporter cell line.

14. The method of either one of claims 10 or 11 wherein said DNA binding domain is selected from the group consisting of the DNA binding domains of GAL4 and LexA.

15. The method of either one of claims 10 or 11 wherein said mammalian cell is human.

16. The method of claim 15 wherein said mammalian cell is a HeLa cell.

17. A method of assaying for the activation of a conditionally active transactivation domain in a mammalian cell, said method comprising the steps of:

detecting in a stable reporter cell line that is subjected to conditions which permit activation of the conditionally active transactivation domain the expression of a reporter gene, the reporter cell line comprising:

a reporter gene operably linked to a recognition sequence for a sequence-specific DNA-binding protein; and

a stably integrated recombinant nucleic acid construct comprising a sequence encoding a fusion protein, said fusion protein comprising a sequence-specific DNA binding domain, wherein said DNA binding domain specifically binds said recognition sequence, and a conditionally active transactivation domain, wherein activation of said conditionally active transactivation domain is dependent on protein phosphorylation and/or protein:protein interaction, wherein binding of said fusion protein to said recognition sequence results in transactivation of said reporter gene when said transactivation domain fused to said DNA binding domain is activated; and wherein expression of said reporter gene is indicative of the activity of said conditionally active transactivating protein.

18. A method of screening for a modulator of the activity of a conditionally active transactivation domain in a mammalian cell, said method comprising the steps of:

(a) contacting a stable reporter cell line with a candidate modulator under conditions sufficient to permit activation of said conditionally active transactivation domain, the reporter cell line comprising:

a reporter gene operably linked to a recognition sequence for a sequence-specific DNA-binding protein; and

a stably integrated recombinant nucleic acid construct comprising a sequence encoding a fusion protein, said fusion protein comprising a sequence-specific DNA binding domain, wherein said DNA binding domain specifically binds said recognition sequence, and a conditionally active transactivation domain, wherein activation of said conditionally active transactivation domain is dependent on protein phosphorylation and/or protein:protein interaction, wherein binding of said fusion protein to said recognition sequence results in transactivation of said reporter gene when said transactivation domain fused to said DNA binding domain is activated; and

(b) detecting the expression of said reporter gene, wherein a difference in expression of said reporter gene in the presence of said candidate modulator and in the absence of said candidate modulator is indicative of modulatory activity of said candidate modulator on said conditionally active transactivating protein.

19. The method of claim 18, further comprising, during said contacting step, providing an activator signal, said signal activating said conditionally active transactivation domain.

20. The method of claim 19, wherein said providing comprises adding an activator compound to the culture medium of said reporter cell line.

21. The method of either one of claims 17 or 18 wherein said DNA binding domain is selected from the group consisting of the DNA binding domains of GAL4 and LexA.

22. The method of either one of claims 17 or 18 wherein said mammalian cell is human.

23. The method of claim 22 wherein said mammalian cell is a HeLa cell.

24. A kit comprising the cell line of claim 1 and packaging therefor.

25. A kit for performing the method of any one of claims 10, 11, 17 or 18, said kit comprising a cell line comprising a stably integrated recombinant nucleic acid construct comprising:

a reporter gene operably linked to a recognition sequence for a sequence-specific DNA-binding protein; and

a stably integrated recombinant nucleic acid construct comprising a sequence encoding a fusion protein, said fusion protein comprising a sequence-specific DNA binding domain, wherein said DNA binding domain specifically binds said recognition sequence, and a conditionally active transactivation domain, wherein activation of said conditionally active transactivation domain is dependent on protein phosphorylation and/or protein:protein interaction, and wherein binding of said fusion protein to said recognition sequence results in transactivation of said reporter gene when said transactivation domain fused to said DNA binding domain is activated.

26. The kit of claim 25, said kit further comprising a nucleic acid expression

construct encoding an upstream activator of the conditionally active transactivation domain.

27. A method of assaying for the interaction of two proteins in a mammalian cell, said method comprising the steps of:

(I) detecting in a mammalian cell expression of a reporter gene, wherein said mammalian cell contains in its genome a stably integrated recombinant reporter gene construct comprising a reporter gene operably linked to one or more copies of a sequence-specific recognition site, and said mammalian cell comprises (a) a recombinant nucleic acid construct encoding a first protein fused to a DNA binding domain that specifically binds said sequence-specific recognition site; (b) a recombinant nucleic acid construct encoding a second protein fused to an activation domain of a transcriptional activator protein, wherein said activation domain transactivates expression of a gene to which it is bound; and

II) detecting the expression of said reporter gene, wherein said expression indicates interaction of said first protein and said second protein in said mammalian cell.

28. A method of screening for a modulator of the interaction of two proteins known to interact in a mammalian cell, said method comprising the steps of:

i) contacting a mammalian cell with a candidate modulator, said mammalian cell containing in its genome a stably integrated reporter gene construct which comprises a reporter gene operably linked to one or more copies of a sequence-specific recognition site, and said mammalian cell also comprises

(a) a nucleic acid construct encoding a first protein of an interaction pair fused to a

DNA binding domain which specifically binds said sequence-specific recognition site; and

(b) a nucleic acid construct encoding a second protein of an interaction pair fused to an activation domain of a transcriptional activator protein, wherein said activation domain transactivates expression of a gene to which it is bound; and

ii) detecting the expression of said reporter gene, wherein a difference in reporter gene expression in the presence and absence of said candidate modulator is indicative of modulation by said candidate modulator of the interaction of said first and second proteins in said mammalian cell.

29. The method of either one of claims 27 or 28 wherein said DNA binding protein or DNA binding domain thereof is selected from the group consisting of GAL4 and LexA.

30. The method of either one of claims 27 or 28 wherein said DNA binding protein or DNA binding domain thereof is GAL4.

31. The method of either one of claims 27 or 28 wherein said mammalian cell is derived from a human.

32. The method of claim 31 wherein said mammalian cell is a HeLa cell.